

SEQUENCE LISTING

<110> Terumo Corporation

<120> Functional Hybrid Polypeptide with Collagen-binding
Activity

<130> 19990120

<140>

<141>

<160> 16

<170> PatentIn Ver. 2.0

<210> 1

<211> 343

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modified Human
Fibronectin Collagen-Binding Domain

<220>

<221> INIT __MET

<222> (1)

<220>

<221> DOMAIN

<222> (2)..(341)

<223> /note="human fibronectin collagen-binding domain"

<220>

<221> CONFLICT

<222> (69)

<220>

<221> CONFLICT

<222> (125)

<400> 1

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Gly	His	Cys	Val	Thr	Asp	Ser	Gly	Val	Val	Tyr	Ser	Val	Gly	Met	Gln
			20						25					30	
Trp	Leu	Lys	Thr	Gln	Gly	Asn	Lys	Gln	Met	Leu	Cys	Thr	Cys	Leu	Gly
			35					40					45		
Asn	Gly	Val	Ser	Cys	Gln	Glu	Thr	Ala	Val	Thr	Gln	Thr	Tyr	Gly	Gly
		50						55					60		
Asn	Ser	Asn	Gly	Glu	Pro	Cys	Val	Leu	Pro	Phe	Thr	Tyr	Asn	Gly	Arg
		65					70				75				80
Thr	Phe	Tyr	Ser	Cys	Thr	Thr	Glu	Gly	Arg	Gln	Asp	Gly	His	Leu	Trp
					85					90				95	
Cys	Ser	Thr	Thr	Ser	Asn	Tyr	Glu	Gln	Asp	Gln	Lys	Tyr	Ser	Phe	Cys
					100					105				110	
Thr	Asp	His	Thr	Val	Leu	Val	Gln	Thr	Arg	Gly	Gly	Asn	Ser	Asn	Gly
			115						120					125	
Ala	Leu	Cys	His	Phe	Pro	Phe	Leu	Tyr	Asn	Asn	His	Asn	Tyr	Thr	Asp
			130				135						140		
Cys	Thr	Ser	Glu	Gly	Arg	Arg	Asp	Asn	Met	Lys	Trp	Cys	Gly	Thr	Thr
			145							150			155		160
Gln	Asn	Tyr	Asp	Ala	Asp	Gln	Lys	Phe	Gly	Phe	Cys	Pro	Met	Ala	Ala
					165					170				175	
His	Glu	Glu	Ile	Cys	Thr	Thr	Asn	Glu	Gly	Val	Met	Tyr	Arg	Ile	Gly
					180					185				190	

Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys Thr
 195 200 205
 Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser Gln
 210 215 220
 Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn Asp
 225 230 235 240
 Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr Cys
 245 250 255
 Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln Cys
 260 265 270
 Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp Glu
 275 280 285
 Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg Gly
 290 295 300
 Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser Ser
 305 310 315 320
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 325 330 335
 His Pro Ile Gln Trp Leu Glu
 340

<210> 2

<211> 159

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Human Basic

Fibroblast Growth Factor with Enterokinase

Recognition Sequence

<220>

<221> PEPTIDE

<222> (1)..(5)

<223> /note="enterokinase recognition sequence"

<220>

<221> PEPTIDE

<222> (6)..(159)

<223> /note="human fibroblast growth factor"

<400> 2

Asp Asp Asp Asp Lys Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu

1 5 10 15

Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp

20 25 30

Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His

35 40 45

Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile

50 55 60

Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly

65 70 75 80

Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu

85 90 95

Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu

100 105 110

Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr

115 120 125

Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly

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Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
145              150              155

<210> 3
<211> 58
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:Human Epidermal
      Growth Factor with Enterokinase Recognition
      Sequence
<220>
<221> PEPTIDE
<222> (1)..(5)
<223> /note="enterokinase recognition sequence"
<220>
<221> PEPTIDE
<222> (6)..(58)
<223> /note="human epidermal growth factor"
<400> 3

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   1               5               10               15
Gly Tyr Cys Leu His Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp
           20               25               30
Lys Tyr Ala Cys Asn Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln
       35               40               45
Tyr Arg Asp Leu Lys Trp Trp Glu Leu Arg
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50

55

<210> 4

<211> 501

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Basic Fibroblast Growth Factor

<220>

<221> INIT __MET

<222> (1)

<220>

<221> DOMAIN

<222> (2)..(341)

<223> /note="human fibronectin collagen-binding domain"

<220>

<221> PEPTIDE

<222> (343)..(347)

<223> /note="enterokinase recognition sequence"

<220>

<221> PEPTIDE

<222> (348)..(501)

<223> /note="human fibroblast growth factor"

<400> 4

Met Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro Tyr

1

5

10

15

Gly	His	Cys	Val	Thr	Asp	Ser	Gly	Val	Val	Tyr	Ser	Val	Gly	Met	Gln
20				25				30							
Trp	Leu	Lys	Thr	Gln	Gly	Asn	Lys	Gln	Met	Leu	Cys	Thr	Cys	Leu	Gly
35				40				45							
Asn	Gly	Val	Ser	Cys	Gln	Glu	Thr	Ala	Val	Thr	Gln	Thr	Tyr	Gly	Gly
50				55				60							
Asn	Ser	Asn	Gly	Glu	Pro	Cys	Val	Leu	Pro	Phe	Thr	Tyr	Asn	Gly	Arg
65				70				75				80			
Thr	Phe	Tyr	Ser	Cys	Thr	Thr	Glu	Gly	Arg	Gln	Asp	Gly	His	Leu	Trp
85				90				95							
Cys	Ser	Thr	Thr	Ser	Asn	Tyr	Glu	Gln	Asp	Gln	Lys	Tyr	Ser	Phe	Cys
100				105				110							
Thr	Asp	His	Thr	Val	Leu	Val	Gln	Thr	Arg	Gly	Gly	Asn	Ser	Asn	Gly
115				120				125							
Ala	Leu	Cys	His	Phe	Pro	Phe	Leu	Tyr	Asn	Asn	His	Asn	Tyr	Thr	Asp
130				135				140							
Cys	Thr	Ser	Glu	Gly	Arg	Arg	Asp	Asn	Met	Lys	Trp	Cys	Gly	Thr	Thr
145				150				155				160			
Gln	Asn	Tyr	Asp	Ala	Asp	Gln	Lys	Phe	Gly	Phe	Cys	Pro	Met	Ala	Ala
165				170				175							
His	Glu	Glu	Ile	Cys	Thr	Thr	Asn	Glu	Gly	Val	Met	Tyr	Arg	Ile	Gly
180				185				190							
Asp	Gln	Trp	Asp	Lys	Gln	His	Asp	Met	Gly	His	Met	Met	Arg	Cys	Thr
195				200				205							
Cys	Val	Gly	Asn	Gly	Arg	Gly	Glu	Trp	Thr	Cys	Ile	Ala	Tyr	Ser	Gln
210				215				220							
Leu	Arg	Asp	Gln	Cys	Ile	Val	Asp	Asp	Ile	Thr	Tyr	Asn	Val	Asn	Asp

225	230	235	240
Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr Cys			
245	250	255	
Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln Cys			
260	265	270	
Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp Glu			
275	280	285	
Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg Gly			
290	295	300	
Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser Ser			
305	310	315	320
Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn Ser			
325	330	335	
His Pro Ile Gln Trp Leu Asp Asp Asp Asp Lys Ala Ala Gly Ser Ile			
340	345	350	
Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro			
355	360	365	
Pro Gly His Phe Lys Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly			
370	375	380	
Phe Phe Leu Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg Glu			
385	390	395	400
Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly			
405	410	415	
Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys			
420	425	430	
Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val Thr Asp Glu Cys Phe			
435	440	445	

Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg

450

455

460

Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys

465

470

475

480

Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro

485

490

495

Met Ser Ala Lys Ser

500

<210> 5

<211> 400

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Epidermal Growth Factor

<220>

<221> INIT __MET

<222> (1)

<220>

<221> DOMAIN

<222> (2)..(341)

<223> /note="human fibronectin collagen-binding domain"

<220>

<221> PEPTIDE

<222> (343)..(347)

<223> /note=" enterokinase recognition sequence"

<220>

<221> PEPTIDE

<222> (348)..(400)

<223> /note="human epidermal growth factor"

<400> 5

Met Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro Tyr

1 5 10 15

Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met Gln

20 25 30

Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu Gly

35 40 45

Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly Gly

50 55 60

Asn Ser Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly Arg

65 70 75 80

Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu Trp

85 90 95

Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe Cys

100 105 110

Thr Asp His Thr Val Leu Val Gln Thr Arg Gly Gly Asn Ser Asn Gly

115 120 125

Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr Asp

130 135 140

Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr Thr

145 150 155 160

Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala Ala

165 170 175

His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile Gly
 180 185 190
 Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys Thr
 195 200 205
 Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser Gln
 210 215 220
 Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn Asp
 225 230 235 240
 Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr Cys
 245 250 255
 Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln Cys
 260 265 270
 Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp Glu
 275 280 285
 Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg Gly
 290 295 300
 Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser Ser
 305 310 315 320
 Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn Ser
 325 330 335
 His Pro Ile Gln Trp Leu Asp Asp Asp Asp Lys Asn Ser Asp Ser Glu
 340 345 350
 Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp Gly Val Cys Met
 355 360 365
 Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val Val Gly Tyr
 370 375 380
 Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu Leu Arg

385	390	395	400
<210> 6			
<211> 49			
<212> DNA			
<213> Artificial Sequence			
<220>			
<223> Description of Artificial Sequence:PCR Sense			
Primer for Human Fibronectin Collagen-Binding			
Domain			
<400> 6			
gaggtacat ggtacatatg gcagctgttt accaaccgca gcctcaccc			49
<210> 7			
<211> 46			
<212> DNA			
<213> Artificial Sequence			
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<223> Description of Artificial Sequence:PCR Antisense			
Primer for Human Fibronectin Collagen-Binding			
Domain			
<220>			
<400> 7			
cgggatacctt actcgagcca ctggatgggg tgggagttgg gctgac			46
<210> 8			
<211> 1053			
<212> DNA			
<213> Artificial Sequence			
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<223> Description of Artificial Sequence: Modified Human
Fibronectin Collagen-Binding Domain

<220>

<221> conflict

<222> (109)

<220>

<221> conflict

<222> (206)

<220>

<221> conflict

<222> (270)

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<221> conflict

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<221> conflict

<222> (681)

<400> 8

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acacaaggaa ataagcaaat gctttgcacg tgcttgggca acggagtcag ctgccaagag 180
acagctgtaa cccagactta cgggtggcaac tcaaatggag agccatgtgt cttaccattc 240
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actgttttgg ttcagactcg aggaggaaat tccaatggtg ccttgtgcca cttccccttc 420
ctatacaaca accacaatta cactgattgc acttctgagg gcagaagaga caacatgaag 480
tggtgtggga ccacacagaa ctatgatgcc gaccagaagt ttgggttctg ccccatggct 540

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 gaatggacat gcattgccta ctgcagctt cgagatcagt gcattgttga tgacatcact 720
 tacaatgtga acgacacatt ccacaagcgt catgaagagg ggcacatgct gaactgtaca 780
 tgcttcggtc agggtcgggg caggtggaag tgtgatcccg tcgaccaatg ccaggattca 840
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<210> 9

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Sense

Primer for Human Basic Fibroblast Growth Factor

<400> 9

gagtcgacga cgatgataag gcagccggga gcatcaccac 40

<210> 10

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Antisense

Primer for Human Basic Fibroblast Growth Factor

<400> 10

ggaattctca gctcttagca gacattggaa g 31

<210> 11

<211> 489

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Human Basic

Fibroblast Growth Factor with Enterokinase

Recognition Sequence

<220>

<221> mutation

<222> (228)

<223> /note="mutation caused by polymerase chain
reaction"

<400> 11

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agcgaccctc acatcaagct acaacttcaa gcagaagaga gaggagtcgt gtctatcaaa 240
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cggatcaagga aatacaccag ttggtatgtg gcactgaaac gaactgggca gtataaactt 420
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tgagaattc                                     489
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<210> 12

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Sense

Primer for Human Epidermal Growth Factor

<400> 12

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<210> 13

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Antisense

Primer for Human Epidermal Growth Factor

<400> 13

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<210> 14

<211> 186

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Human Epidermal

Growth Factor with Enterokinase Recognition

Sequence

<400> 14

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gttggtctaca tcggggagcg atgtcagtac cgagacctga agtggtggga actgcgctaa 180
gaattc 186

<210> 15

<211> 1527

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Fibroblast Growth Factor

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acacaaggaa ataagcaaat gctttgcacg tgccctgggca acggagtcag ctgccaagag 180
acagctgtaa cccagacitc cgggtggcaac tcaaatggag agccatgtgt cttaccattc 240
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gcccacgagg aaatctgcac aaccaatgaa ggggtcatgt accgcattgg agatcagtgg 600
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gaatggacat gcattgccta ctgcagctt cgagatcagt gcattgttga tgacatcact 720
tacaatgtga acgacacatt ccacaagcgt catgaagagg ggcacatgct gaactgtaca 780
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taccagtgtc actgctatgg ccgtggcatt ggggagtggc attgccaacc tttacagacc 960
tatccaagct caagtgttcc tgtcgaagta tttatcactg agactccgag tcagcccaac 1020
tcccacccca tccagtggct cgacgacgat gataaggcag ccgggagcat caccacgtg 1080

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 gacgggggtcc gggagaagag cgaccctcac atcaagctac aacttcaagc agaagagaga 1260
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 aataactaca atacttaccg gtcaaggaaa tacaccagtt ggtatgtggc actgaaacga 1440
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<210> 16

<211> 1224

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Epidermal growth factor

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 acacaaggaa ataagcaaat gctttgcacg tgcttgggca acggagtcag ctgccaagag 180
 acagctgtaa ccagactta cgggtggcaac tcaaatggag agccatgtgt cttaccattc 240
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 tgggtgcagca caacttcgaa ttatgagcag gaccagaaat actctttctg cacagaccac 360
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 ctatacaaca accacaatta cactgattgc acttctgagg gcagaagaga caacatgaag 480
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 gccacagagg aaatctgcac aaccaatgaa ggggtcatgt accgcattgg agatcagtgg 600

gataagcagc atgacatggg tcacatgatg aggtgcacgt gtgttgggaa tggtcgtggg 660
gaatggacat gcattgccta ctgcagctt cgagatcagt gcattgttga tgacatcact 720
tacaatgtga acgacacatt ccacaagcgt catgaagagg ggcacatgct gaactgtaca 780
tgcttcggtc agggtcgggg cagggtggaag tgtgatcccg tcgaccaatg ccaggattca 840
gagactggga cgttttatca aattggagat tcatgggaga agtatgtgca tgggtgtcaga 900
taccagtgtc actgctatgg ccgtggcatt ggggagtggc attgccaacc ttacagacc 960
tatccaagct caagtgttc tgctgaagta ttatcactg agactccgag tcagcccaac 1020
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